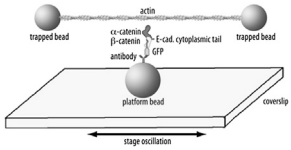
Genetic studies identified the AJ cell-cell adhesion complex of E-cadherin, beta-catenin, and the actin-binding protein alpha-catenin as a minimal ternary complex required for interactions with the actin cytoskeleton. However, attempts to reconstitute in vitro a direct linkage between the cadherin-catenin complex and actin filaments in bulk pelleting assays have been unsuccessful. Contemporary experiments in living cells indirectly showed that force, which was not reconstituted in vitro, is important for normal cell-cell adhesion. As a result, we developed a novel optical trapping assay to determine if and how the cadherin-catenin complex binds to actin filaments under mechanical load (see figure). We find that multiple cadherin-catenin complexes can together form a robust, load-resistant connection to actin that can withstand >10 pN of force for several seconds. Individual cadherin-catenin complexes also bind actin, but the interaction is relatively weak and transient. To our knowledge, these are the first data showing direct, mechanically robust binding between the cadherin-catenin complex and actin filaments. Our results provide essential support to models proposing that the AJ is a mechano-sensitive complex.



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B-Spectrin and the Mechanical Control of the Sense of Touch

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Many somatosensory neurons have evolved specialized molecular sensors that convert mechanical stress into behavioral responses. The genetics, development and physiology of the touch receptor neurons (TRNs) in Caenorhabditis elegans nematodes are especially well characterized and this animal has the particular advantage that the TRNs can be studied both in living animals and dissociated in culture. Like other somatosensory neurons, the TRNs use ion channels to convert mechanical stress into electrical signals and, ultimately, into appropriate behaviors. Whereas the protein partners that form these channels have been known for some time, the nature of the molecular machine important for efficient force transmission from skin to touch receptor neurite is essentially unknown.

Using a combination of approaches, we will show that sensation of mechanical forces depends on a continuous, pre-strained spectrin cytoskeleton inside neurons. We observed that mutations in the tetramerization domain of C. elegans β-spectrin (UNC-70), an actin-membrane crosslinker, lead to defective neuron morphologies under compressive stresses in moving animals. We performed AFM force spectroscopy experiments on isolated neurons, laser axotomy and FRET imaging to measure forces across single cells and molecules. Our data indicate that spectrin is held under constitutive tension in living animals, which contributes to an elevated pre-stress in TRNs. Based on these results and data obtained from optogenetic and mechanical stimulation on β-spectrin mutants, we suggest that β-spectrin-dependent pre-tension is required for efficient responses to external mechanical stimuli.

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A New Model System to Explore the Mechanisms and Functions of Global Cortical Contraction Waves in Oocyte and Embryo Cell Divisions

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Cortical contractions are observable in cell divisions in many species, from meiotic divisions in Ascidian and starfish eggs to the early mitotic divisions of the Xenopus embryo. There have been many hypotheses concerning the function of these contractions, from generating pressure gradients to localizing mRNAs. Importantly, the physical and molecular mechanisms underlying these global contractions remain unknown, but would be essential in providing insights into their functions.

As a system well-suited for live imaging and physical manipulations, we are studying the prominent contraction that occurs at the highly asymmetric meiotic division in starfish oocytes. In this system, the contraction occurs immediately before the division but its importance for the division remains controversial. Therefore, we set out to understand the molecular details and mechanics of the cortical contraction.

Detailed analysis of the oocytes' shape and cytoplasmic flows revealed that the contraction wave is a flattening of the cell cortex which progresses across the whole oocyte towards the site of division with increasing intensity. This results in a strong and varied cytoplasmic flow. Visualizing the localization of components of the cytoskeleton indicates that F-actin and non-muscle myosin II are recruited to the cortical area currently undergoing contraction.

To test the involvement of myosin II in the contraction, we applied the myosin II inhibitor blebbistatin that strongly hindered the contraction wave. Our analysis of the inhibition phenotype will reveal important details of the mechanisms of the contraction wave and allow us to determine the contraction waves' function. We will search for additional factors controlling the contraction wave for which an important clue is that changing the nuclear position prior to contraction repositions the axis of contraction, suggesting the involvement of nuclear factors.

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Barotaxis in a Confined Neutrophil

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Cells integrate multiple measurement modalities to navigate their environment. Soluble and substrate-bound chemical gradients and physical cues have all been shown to influence cell orientation and migration. Here we investigate a novel form of directional sensing, the response to hydraulic pressure, or barotaxis. Cells confined in bifurcating microchannels identified and migrated toward the path of least hydraulic resistance in the absence of chemical cues. The percentage of cells which migrated toward the lower resistance channel increased with increasing resistance ratios. In a bifurcating channel with asymmetric hydraulic resistances, we observed that the cell elaborated two leading edges with the pseudopod along the less resistive channel exhibiting a faster extension rate. Measurements of the bulk fluid flow in a channel containing a migrating cell show that cells must push the column of water in front of them, suggesting that the observed bias in extension rates is due to the different forces experienced by each leading edge. The pressure differences resulting from asymmetrical resistances, were small, suggesting weak force generation by leading edges. The bifurcating microchannel design allowed us to investigate the effect of picoNewton scale forces on leading edge protrusion, whereas previous studies have focused on nanoNewton scale forces. Through the use of a photocaged analogue of the chemokine formyl-methionyl-leucyl-phenylalanine (fMLP), we found that barotaxis was able to override a dynamically generated chemical cue. Motile cells may use barotaxis in concert with chemotaxis to navigate complex environments.

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Cortical and Cytoskeletal Structural Network regulates the Three-Dimensional Traction Forces Exerted by Migrating Amoeboid Cells

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Cells exert traction forces, which are necessary to perform their migration. There is still a lack of information regarding the mechanical aspects of cell migration and in particular the quantification of the three-dimensional traction forces exerted during migration and invasion.

We use a novel traction force cytometry method to measure the three-dimensional traction forces exerted by Dictyostelium cells chemotaxing over flat elastic substrates. Our measurements of the 3-D traction forces show that there are two independent mechanisms in their generation, one that relies on the cytoskeletal crosslinking and contractility, and another based on the cortical stiffness. In particular, we examine the roles that specific cortical and cytoskeletal crosslinking proteins and those liking the cell's cortex and the cytoskeletal F-actin play in the development of the spatial and temporal distribution of three-dimensional traction forces during migration. We investigate mutants with cortical integrity defects, such as cells lacking the protein cortexillin to study the cortical function. We use mutants with cytoskeletal crosslinking defects, such as cells lacking the proteins Myosin II and Abp120 to quantify the function of the cytoskeletal crosslinking. In addition we examine the proteins Myosin IA and B, regarding their role liking the cell's cortex and the cytoskeletal F-actin network.

The effects on the magnitude and distribution of the tangential and perpendicular components of the traction forces to the substrate are shown to be different depending on the specific cytoskeletal and cortical mutations. Our 3-D traction measurements indicate that the vertical and horizontal traction forces are regulated by the cortical and cytoskeletal crosslinking. These two mechanisms are interconnected since the time evolution of the vertical and horizontal forces is well correlated.